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Human CD4$^+$ T-Cell Recognition of Influenza A Virus Hemagglutinin after Subunit Vaccination

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We have examined human CD4$^+$ T-cell recognition of influenza A/Beijing/32/92 (H3N2) virus hemagglutinin following influenza virus HANA subunit vaccination. CD4$^+$ T-cell repertoires were dominated by recognition of epitopes located in conserved regions of the molecule, in a major histocompatibility complex class II haplotype-dependent manner, analogous to that observed following natural infection.

Influenza A virus remains a major cause of human morbidity and mortality. Influvac, an influenza virus HANA subunit vaccine, is widely used for immunoprophylaxis. It is safe and induces satisfactory levels of neutralizing antibodies (11). However, because of continual antigenic variation in virus coat glycoproteins (4, 13, 14), protection by neutralizing antibodies is generally short lived. Subunit influenza virus vaccines are poor inducers of human CD8$^+$ T-cell responses (10), and until now there has never been a systematic investigation of human CD4$^+$ T-cell responses to influenza virus following any form of influenza virus vaccination. CD4$^+$ T cells play a crucial role in the control of influenza virus infection, as the production of neutralizing antibody, immunoglobulin class switching, and affinity maturation are all CD4$^+$ T-cell dependent (1, 6, 12). In addition, CD4$^+$ T cells amplify CD8$^+$ T-cell cytotoxic responses (3, 5, 9) and may participate in viral clearance more directly by the secretion of gamma interferon (2).

We recently demonstrated that in the polymorphic human population, adult CD4$^+$ T-cell repertoires to hemagglutinin (HA) at 3 to 6 months following natural infection with influenza virus A/Beijing/32/92 (H3N2)-like strains are dominated by the recognition of regions of the HA1 and HA2 subunits that are highly conserved among human influenza A virus H3 isolates and that this results in cross-reactive recognition of

TABLE 1. Donor details and expansion of CD4$^+$ T cell lines in vitro

Donor	Age (yr)	Sex[a]	Influenza episode in preceding 4 years[b]	Haplotypes		Cell yield[c]	
				MHC class I	MHC class II	Prevaccination	Post-vaccination
A	31	F	No	A3,23; B7,44; C4,0702	DRB1*1501,0701; DRB4*01; DRB5*01; DQB1*02,0601/2	2.6	4.4
B	34	M	No	A26,31; B38; C0701,1203	DRB1*0301/4,1302; DQB1*06	1.2	7.2
C	31	M	No	A2,66; B27,51; C1,7	DRB0103,080X; DQB1*0301,04	0.7	17.5
D	28	M	No	A2,29; B44,35; C4,1601	DRB1*0101,0701; DRB4*01; DQB1*02,05	1.7	10.7
E	33	M	No	A2,3; B65,51; C5,0802	DRB1*0101,1301; DRB3*01/02/03; DQB1*0501,0603	1.7	10.7
F	42	M	No	A2,31; B51,60; C4,10	DRB1*0101,0402; DRB4*01; DQB1*0302,05	0.6	5.5
G	55	M	—	A2,30; B44,51	DRB1*1501,0701; DRB4*01 DRB5*01; DQB1*02,0601/2	8.1	7.4
H	40	F	Yes	A2; B15,65; C3,10	DRB1*100X,1302; DRB3*01/02/03; DQB1*05,0604-8	13.4	17.4

[a] F, female; M, male.
[b] —, donor G, on the basis of our previous results, is likely to have had a recent subclinical infection (see the text). Donors B, F, and G had received influenza virus vaccines more than 5 years previously.
[c] Cell yield is the ratio of cells recovered at day 21 in culture to the number of peripheral blood mononuclear cells initially seeded: (number of cells recovered on day 7/number of peripheral blood mononuclear cells originally seeded) × (number recovered on day 14/number seeded on day 7) × (number recovered on day 21/number seeded on day 14).

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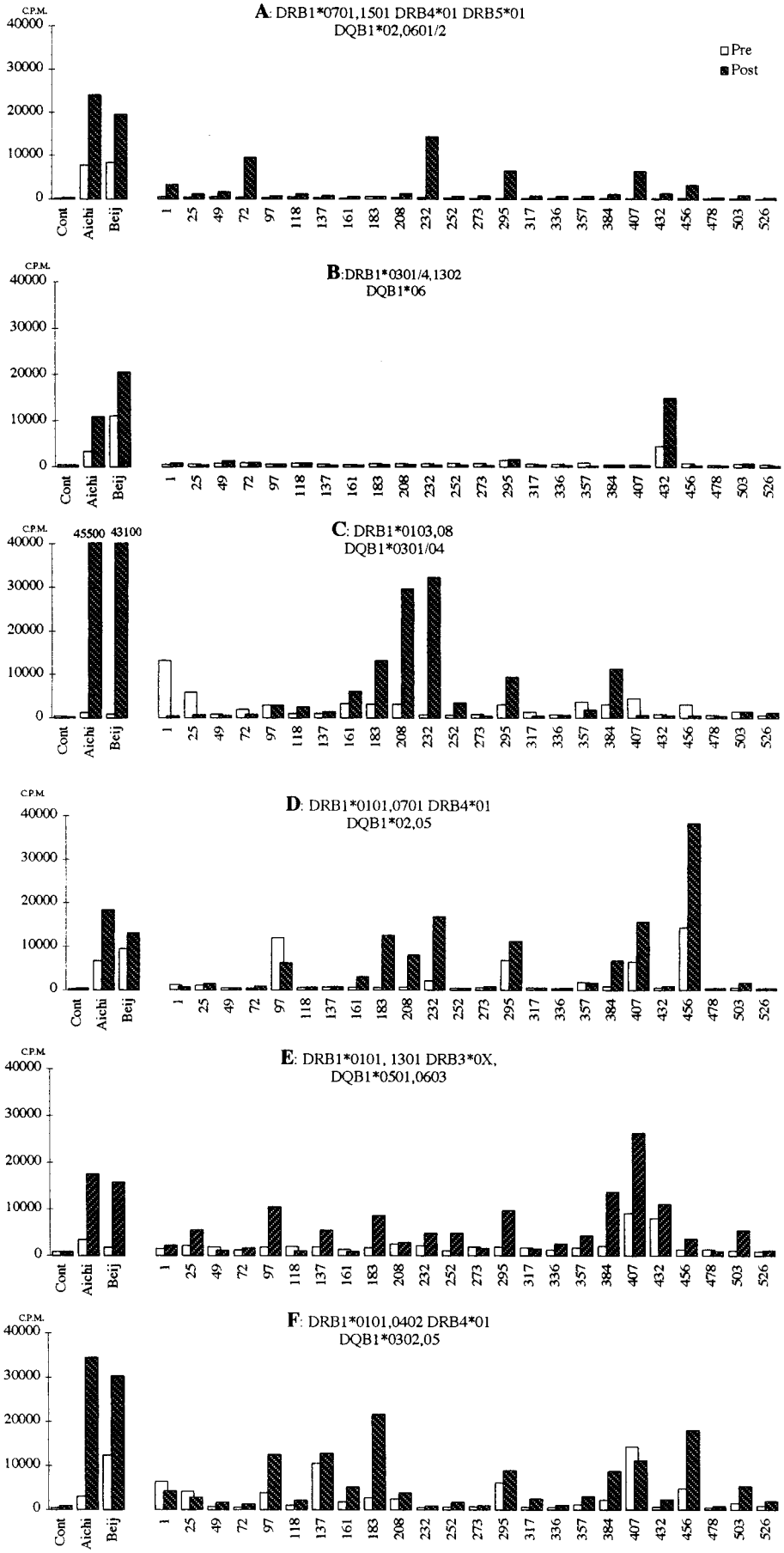
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DQB1*02,0601/2
Pre
Post
B:DRB1*0301/4,1302
DQB1*06
C: DRB1*0103,08
DQB1*0301/04
45500
43100
D: DRB1*0101,0701 DRB4*01
DQB1*02,05
E: DRB1*0101, 1301 DRB3*0X,
DQB1*0501,0603
F: DRB1*0101,0402 DRB4*01
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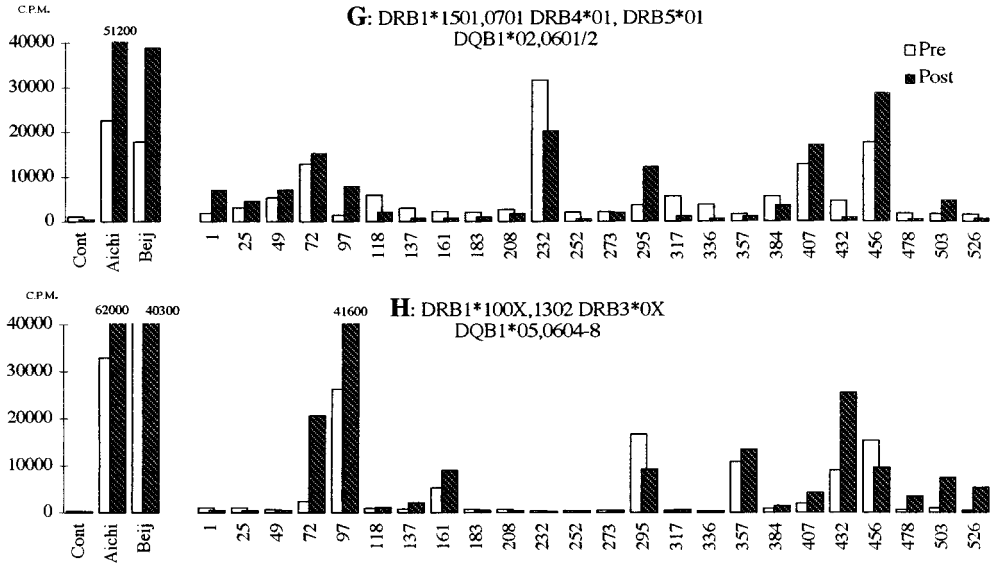

FIG. 2. HA responses by $CD4^+$ T-cell lines derived from donors G and H. Donor H had a history of influenza during December 1993, and donor G, on the basis of our previous results, may have had recent subclinical influenza. The results are shown for samples obtained before (open columns) and 3 to 4 months following (striped columns) vaccination with Influvac. For details, see the legend to Fig. 1.

other human influenza A viruses (8). These findings prompted us to investigate $CD4^+$ T-cell recognition of HA following virus subunit vaccination. In order to allow a direct comparison between the studies, we vaccinated eight unrelated, major histocompatibility complex (MHC)-typed, adult donors with a subunit influenza virus vaccine containing A/Beijing/32/92. HA-specific $CD4^+$ T-cell lines were derived before and at 3 to 4 months following subunit vaccination. Details about the donors are given in Table 1. Donors A to G had no history of influenza for at least 5 years (though donor G may have had a recent subclinical influenza virus infection [see below]). Donor H had a history of influenza during December 1993. Subjects received 0.5 ml of Influvac, containing 15 μg each of HA from influenza A/Beijing/32/92 and A/Singapore/6/86 (H1N1) viruses and influenza B/Panama/45/90 virus, according to the manufacturer's instructions. All other experimental details have been previously described (8). In brief, $CD4^+$ T-cell lines (75, 88% $CD4^+$) were selected for 3 weeks with a full-length HA sequence of influenza A/Beijing/32/92 virus. Detailed HA recognition was examined at days 14 and 21 with a panel of 16 amino acid peptides, overlapping by 11 residues, spanning the entire sequence of the A/Beijing/32/92 HA. Peptide proliferative assays were initially performed with pools of five peptides, and then responses were further localized with individual peptides. Peptides are numbered according to the N-terminal amino acid residue. Peptide pools are numbered by the N-terminal amino acid of the N-terminal peptide. Possible cross-reactivity among H3 influenza A virus HAs was investigated with HA derived from A/Aichi/68 (H3N2).

Prior to subunit vaccination, $CD4^+$ T-cell lines derived from donors A to F expanded 0.6- to 2.6-fold in the presence of antigen (Table 1) and had A/Aichi/68 and A/Beijing/32/92 HA-specific proliferative responses of less than 12,000 cpm (Fig. 1). Two donors (C and E) mounted negligible responses to HA. In marked contrast, $CD4^+$ T-cell lines derived from donors G and H expanded at least eightfold during culture and had higher levels of HA-specific proliferation (Fig. 2). Donor H had a history of influenza during November and December of 1993, whereas donor G had no recollection of an influenza-like illness during the past 5 years. We have previously reported a marked difference in the relative in vitro expansion of $CD4^+$ T-cell lines derived from donors recently exposed to influenza (7.2-fold expansion during 21 days of culture) compared with those from adults with no recent history of influenza (1.1-fold expansion during 21 days of culture) (8). We therefore believe that donor G had a recent subclinical influenza virus infection.

Following vaccination, $CD4^+$ T-cell lines derived from donors A to F showed increases in both in vitro cell expansion (Table 1) and A/Beijing/32/92 and A/Aichi/68 HA-specific proliferation (Fig. 1). Vaccination clearly boosted the proliferative responses of donors A to F to the HA peptide pools (Fig. 1). Serum from each donor recognized at least one of the pools representing the highly conserved HA2 subunit (residues 329 to 550). Furthermore, the majority of responses to the HA1 subunit localized to regions conserved among influenza A virus H3 strains (individual peptide data not shown), with only two major exceptions: donor E responded to residues 146 to 61, which included one conservative substitution between A/Aichi/68 and A/Beijing/32/92 (G-146 to S) and five nonconservative substitutions (T-155 to H, E-156 to K, G-158 to E, S-159 to Y, and T-160 to K), and donor F responded to residues 137 to 157, which included four conservative substitutions (S-143 to P,

FIG. 1. $CD4^+$ T-cell lines were derived from donors A to F before (open columns) and 3 months following (striped columns) vaccination with Influvac. Cell lines were selected with the full-length HA sequence from influenza A/Beijing/32/92 (H3N2) virus. T-cell proliferation responses to A/Aichi/68 (H3N2) HA (1.0 μg/ml), A/Beijing/32/92 HA (0.1 μg/ml), and peptide pools (five peptides per pool; 10 μg of each peptide per ml) were tested at 14 or 21 days of culture with irradiated autologous prepulsed peripheral blood mononuclear cells as antigen-presenting cells. At 48 h, T-cell lines were pulsed with [^{3}H]thymidine and harvested 16 h later. The *x* axis represents the geometric mean of triplicate wells. Control (Cont), proliferative response of T-cell lines to complete medium and autologous antigen-presenting cells; Aichi, response of T-cell line to A/Aichi/68 HA (1.0 μg/ml); Beij, response to A/Beijing/32/92 HA (0.1 μg/ml). Peptide pools are labelled according to the number of the N-terminal residue of the N-terminal peptide in the pool (five peptides per pool; 10 μg of each peptide per ml).

S-145 to N, G-146 to S, and T-155 to H) and three nonconservative substitutions (N-137 to Y, G-144 to V, and E-156 to K) (7).

Donors G and H showed no marked increase in the cell expansion of their $CD4^+$ T-cell lines following vaccination. The magnitudes of their anti-A/Aichi/68 and anti-A/Beijing/32/92 HA-specific proliferative responses did increase but were associated with a much smaller overall rise in the A/Beijing/32/92 HA-specific peptide response. In both donors, the HA2 subunit was recognized, and the great majority of their responses to HA1 were directed to conserved regions.

As the vaccine contained HA derived from an H1 influenza A virus, we were not able to directly examine the cross-reactivity of response with H1 and H2 influenza A viruses. However, because donors A, D, F, G, and H responded to residues 463 to 473 of HA and donors B and E responded to residues 442 to 462 and both regions are highly conserved among human influenza A virus subtypes, such cross-reactivity is likely.

Following natural infection we identified two conserved regions of HA, residues 303 to 323 and 407 to 428, which induced $CD4^+$ T-cell responses from 17 of 18 unrelated donors, despite differences in their MHC class II haplotypes (8). Following subunit vaccination, these regions were recognized by 8 of 8 and 6 of 8 donors, respectively, though the magnitudes of individual donors' responses varied.

Clear MHC class II haplotype-associated recognition patterns of HA were evident. Donors A and G, who express identical DRB1* and DQ1* alleles, responded to the same peptide pools, despite differences in their prevaccination responses. Furthermore, donors D, E, and F, who share the DRB1*0101- and DQB1*05-containing MHC class II haplotype, responded to peptide pools 97, 183, 295, and 407. Our study of natural infection (8) included several donors with this haplotype, allowing a direct comparison. For the two groups, identical peptide pools were recognized, and the responses within these pools were localized to identical individual peptides or pairs of peptides (data not shown). Overall, the levels of proliferative response by the two groups were similar. We are currently investigating whether these results also reflect similar patterns of cytokine production.

In conclusion, influenza virus subunit vaccination enhances $CD4^+$ T-cell recognition of conserved HA epitopes in a manner analogous to that observed following natural infection. At present influenza virus vaccines are offered to high-risk groups on an annual basis. It will therefore be important to monitor $CD4^+$ T-cell responses after repeated annual vaccinations and to define the optimal interval between vaccinations for the maintenance of strong $CD4^+$ T-cell responses to conserved HA epitopes.

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